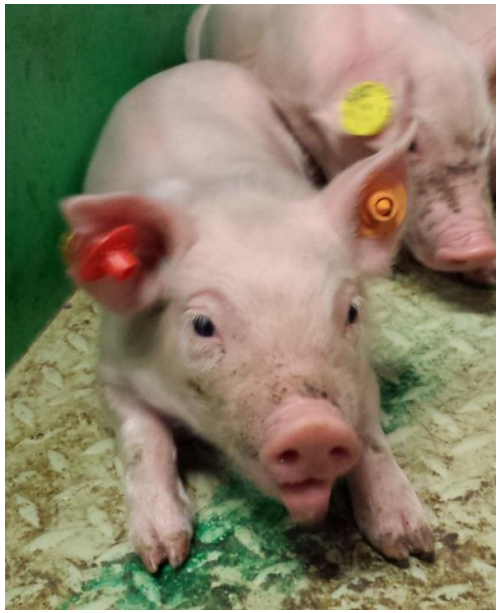
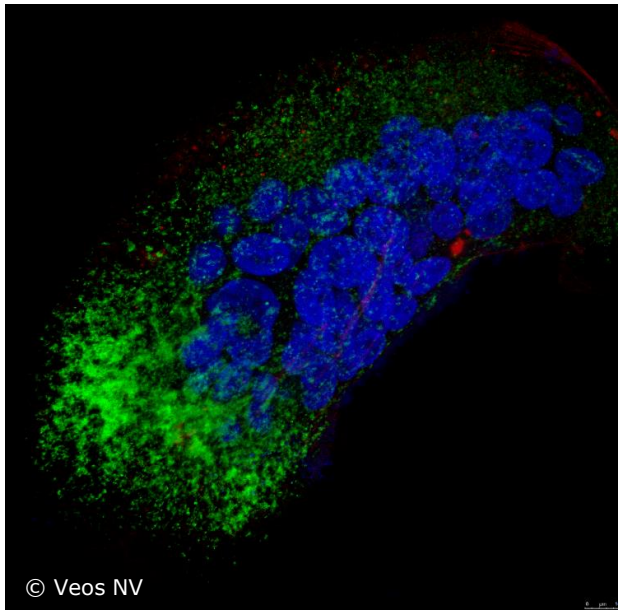


piglet feed

Non-specific antiviral components in plasma can contribute to the safety of SDPP towards PEDV



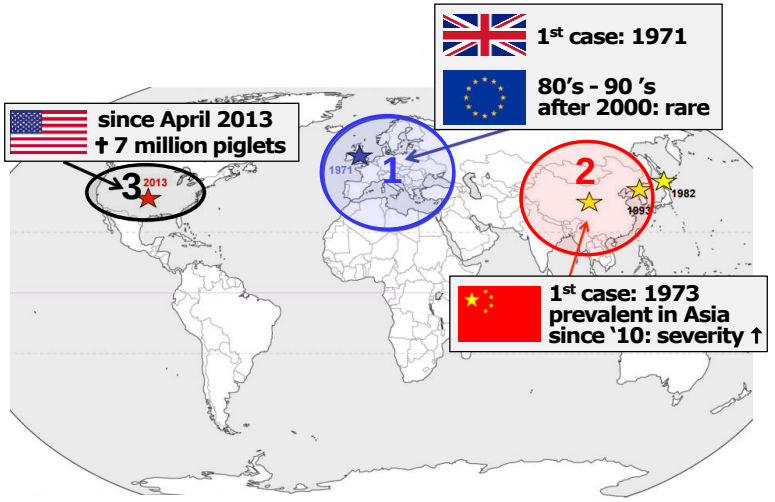


Kalmar ID[1], *Nauwynck HJ*[2], *Quist-Rybachuk GV*[1]

[1]*Veterinary R&D, Veos group, BE*
[2]*Laboratory of Virology, Ghent University, BE*

Background - Porcine Epidemic Diarrhea Virus (PEDV)

- swine alfa-corona virus
- major infection route: Faecal-Oral
- highly enteropathogenic
  - ➜ small intestinal villus atrophy
  - ➜ acute, watery diarrhea (malabsorptive & maldigestive)



After Opriessnig, 2014

- Severity strongly depends on age at infection

(also on virus strain, lactogenic immunity, co-infections, ...)

Neonatal & Suckling	Weanling to Adult	Gestation
➡ up to 80-100 M% ○ fetid, watery diarrhea ○ anorexia ○ vomiting	➡ self-limiting (mild) ○ diarrhea ○ depression ○ loss of apetite ○ weight loss ○ (vomiting)	➡ Reproduction ↓ ○ FR ↓ - RR ↑ - AR ↑ ○ TB ↓ - BA ↓ - Mu ↑ ○ especially gilts, d1-d30 (Orlanratmanee *et al.*, 2010)

Background - Transmission of PEDV

- **Faecal-Oral**
  - **Direct** — **Infected pigs**
  - **Indirect** — **Faecally contaminated fomites**
    equipement, clothing, lorries,…

Live pig collection points (USA July '14)
6,6% of trailers: RNA-pos at arrival
5,2% of RNA neg trailers: left RNA-pos
(Lowe et al., 2014)

? Milk-borne
lactating sows

RNA detected in sow milk
Infectivity not tested
(Sun et al, 2011; Li et al, 2011; Chen et al, 2013)

? Aerosolised
- faeces
- vomit
- saliva

RNA in air samples 16 km downwind infected farms
Field samples : Not infectious
Exp. samples : Infectious
(Alonso et al., 2014)

Background - Transmission of PEDV

- **Faecal-Oral**
  - **Direct** — **Infected pigs**
  - **Indirect** — **Faecally contaminated fomites**
    equipement, clothing, lorries,…

FEED - INGREDIENTS

Proven field cases (USA Jan '14)

- 3 breeding herds: "emergency feed"
- Origin diets: different mills
- clinical sings 2 d post-delivery

- Ingredients: corn - soybean meal - Vit&Min

➡ Ct feed = 19,5-22,2
➡ Bioassay: infectious PEDV

(Dee et al., 2014)

Adequate biosafety measures should be in place for feed and its ingredients

Background - What about SDPP ?

- **Ontario-cases, 2014**
  - **Feed : common factor (Ct 37-40)**
  - **SDPP : Ct 36-37 (Ct 37-40)**

US-SDPP

bioassay CFIA : POS (sampled at feedmill) (Pasick et al., 2014)
bioassay US FDA : NEG (sampled at production plant) (US FDA, 2014)

- **Ontario-cases occured > 10 weeks post-production of SDPP**

Epidemiology **Brazil & West-Canada remained neg** (Crenshaw et al., 2014)
Despite large imports of PCR-pos US-SDPP
(2.5-3.5 M Brazilian pigs/3.5-4.0 M West-Canadian pigs)

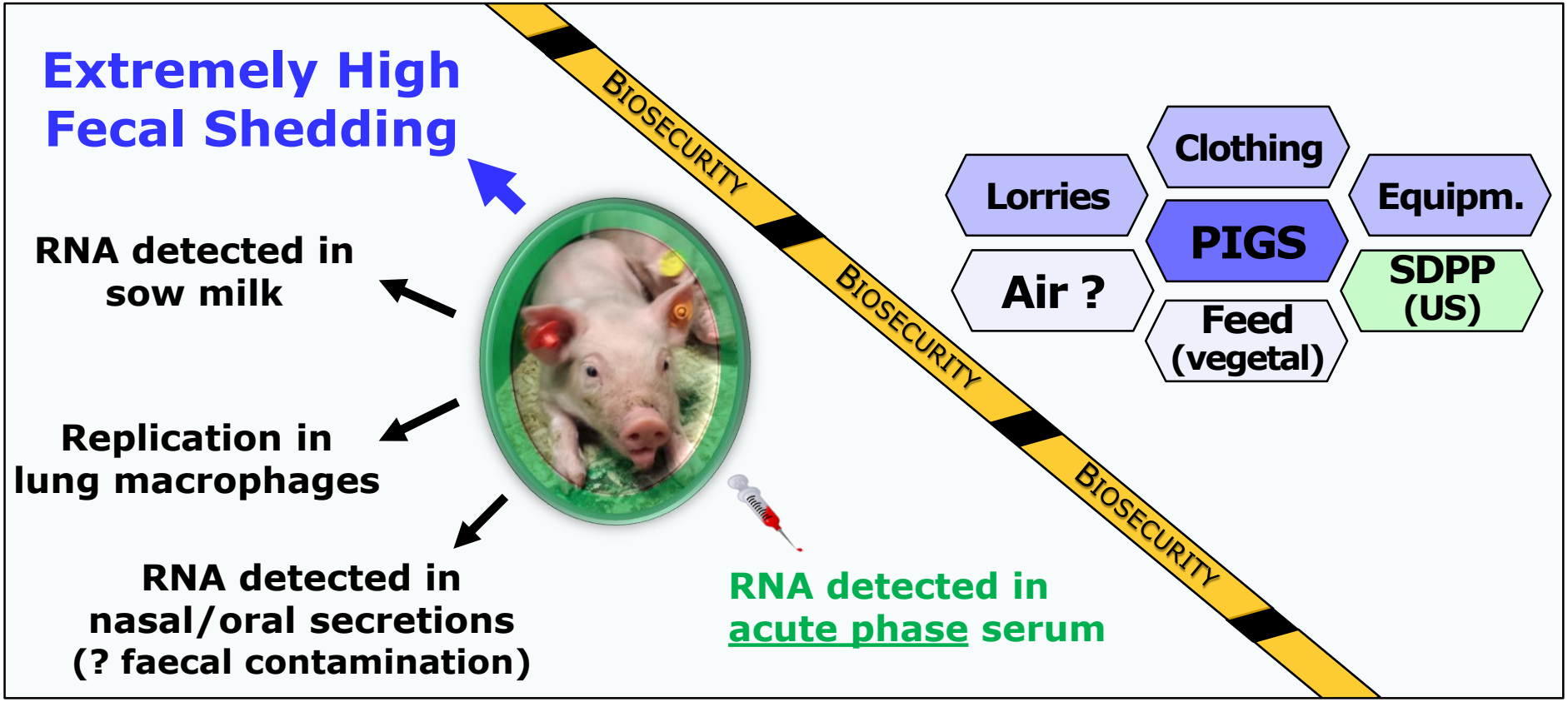
Timing **PEDV is sensitive to dessication** (Pujols & Segalés, 2014)
Inactivation of 2.8 $\log_{10}$ $TCID_{50}$/g (Ct 22)
3 wk at 4°C (Ct 31.5) ; 2 wk at 12°C (Ct 24); 1 wk at 21°C (Ct 21)

Processing **PEDV is sensitive to spray-drying** (Gerber et al, 2014; Pujols & S, 2014)
Inactivation of 4.2 $\log_{10}$ $TCID_{50}$/ml (Ct 13.9)
Spraydrying at 80 °C or 70 °C outlet temp (Ct 23.3-23.8)

Present Trial

Why ?

- Prevention of further geographical spread of PEDV is critical
- Adequate biosafety measures should be in place for feed and feed ingredients, including SDPP



Present Trial

Research Questions

1. **Does porcine plasma contain non-specific anti-PEDV activity ?**

   - **3-5 dpi : 2.3-3.2 log PEDV ge/ml serum** (Opriessnig et al. 2014)
   - **Bio-assay: 3-5 dpi, seronegative serum (body temp) : non-infectious** (Gerber et al., 2014)

---

2. **Are anti-PEDV effects in plasma temperature dependent ?**
   [refrigerated plasma (4°C) vs living pig (37.8-40°C°)]

   - **Bio-assay: PEDV spiked, seronegative serum (4°C) : infectious** (Gerber et al., 2014) (but high spike)

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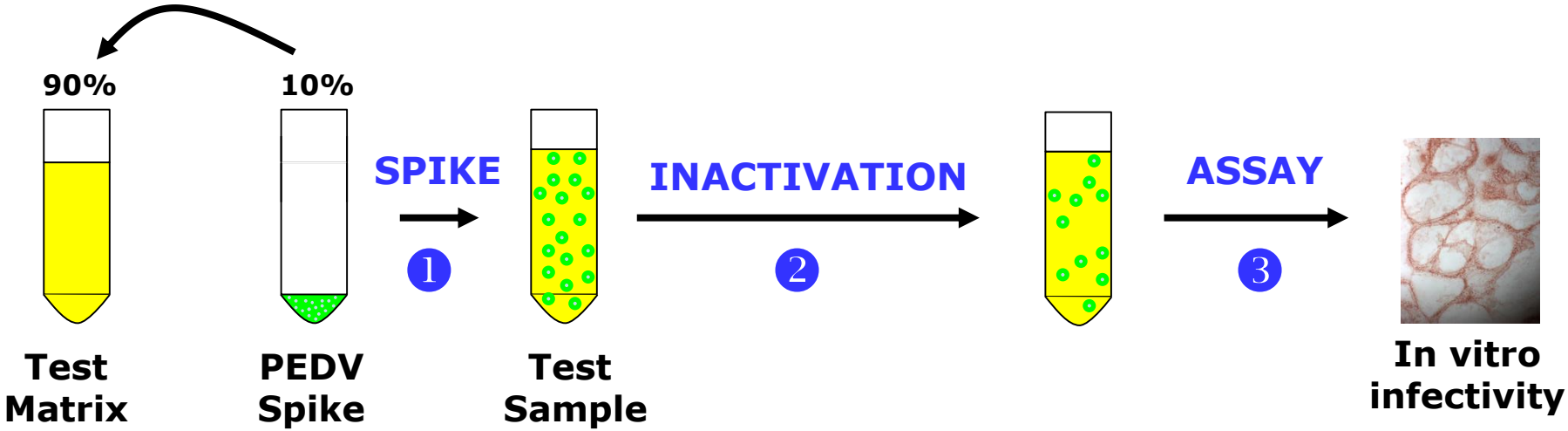
3. **Is PEDV inactivation facilitated in porcine plasma ?**

   - **ingredient-specific PEDV survival at outdoor storage (-25 to 20 °C)**

     SDPP, VTM, soy oil, corn : ≤ 7 d
     DDGS, M&B, Lys, stock-virus : > 30 d
     Soybean meal : >180 d (≤210 d)

     ⇨ **non-infectious (in vitro and in bio-assay)** (Dee et al., 2015)

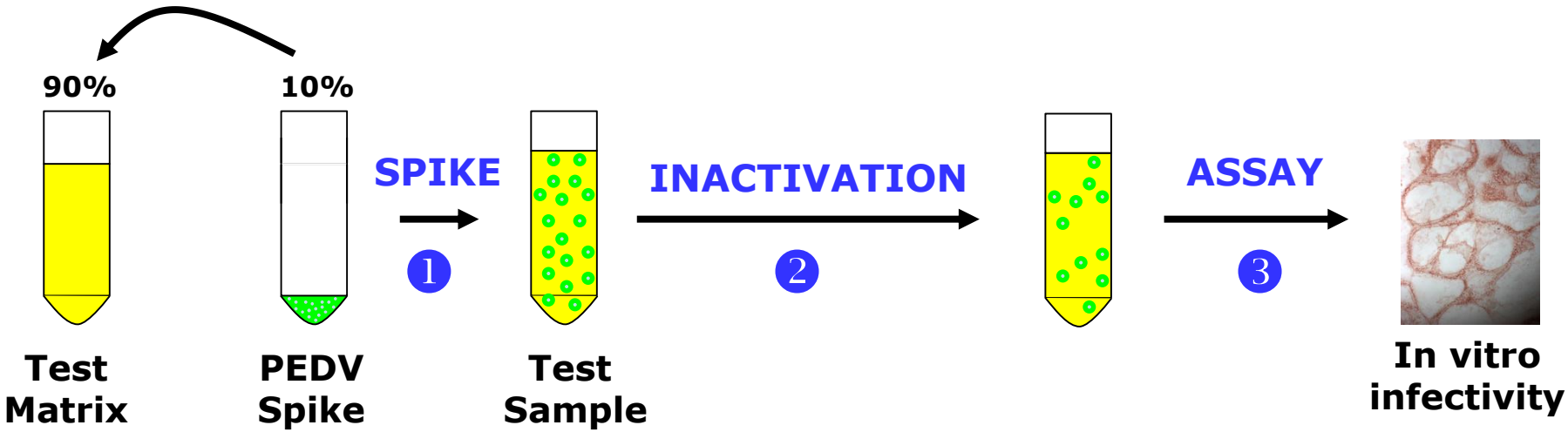
Materials & Methods - Spike Inactivation Assays



❶ **90:10 matrix (MEM or porcine plasma):virus**

sterile filtered
heat inactivated
seronegative for PEDV

Materials & Methods - Spike Inactivation Assays



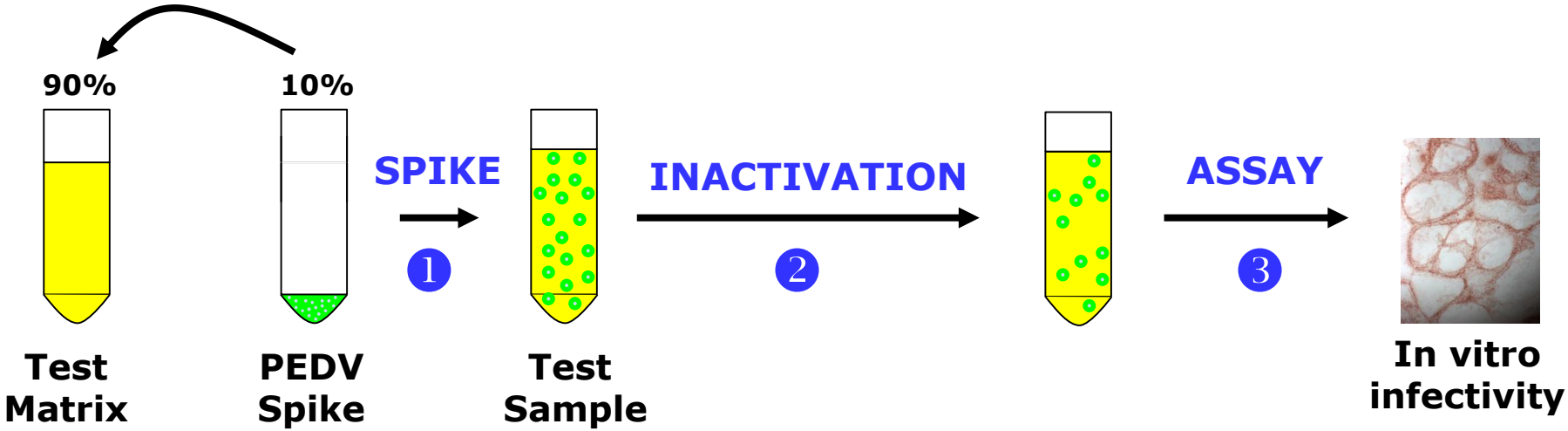
❷ Inactivation (treatment)

- **Condition**
  pH: neutral (pH 7.2)
  temperature: 4, 37 or 40 °C (body temp: 37.8 - 40 °C)

- **Time**
  up to 180 min

- **Matrix**
  MEM or porcine plasma

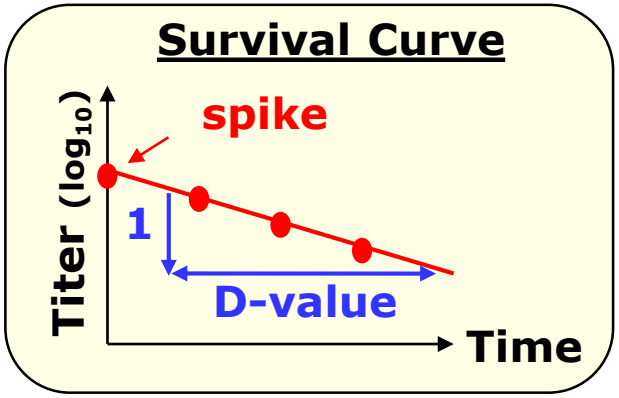
Materials & Methods - Spike Inactivation Assays



❸ **virus titration of whole test sample**

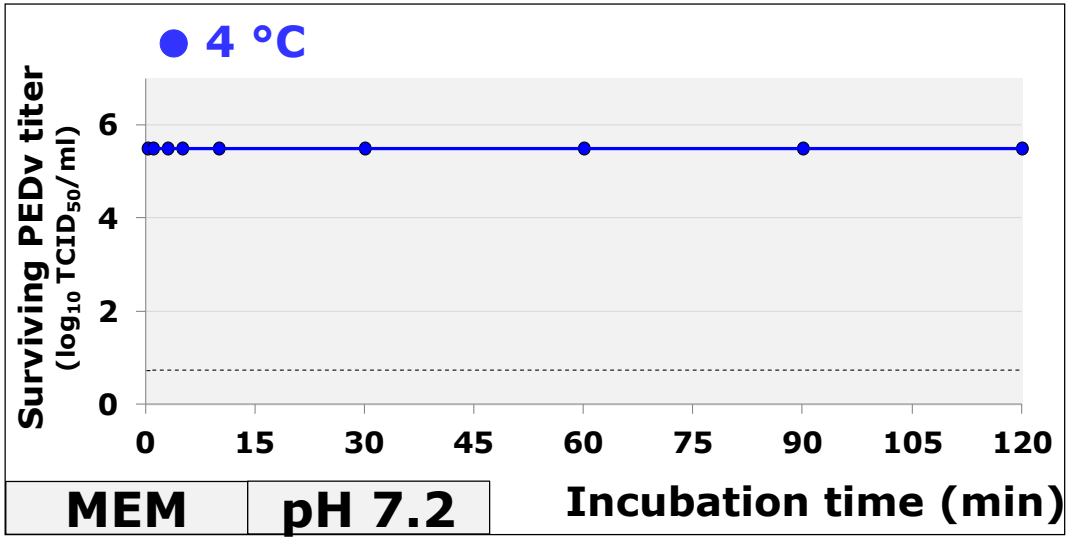
- **100 ul test samples**
  - ➡ **$TCID_{50}$ in 96-well plates (survival curves, n=3)**
- **1 ml test samples**
  - ➡ **PFU count**

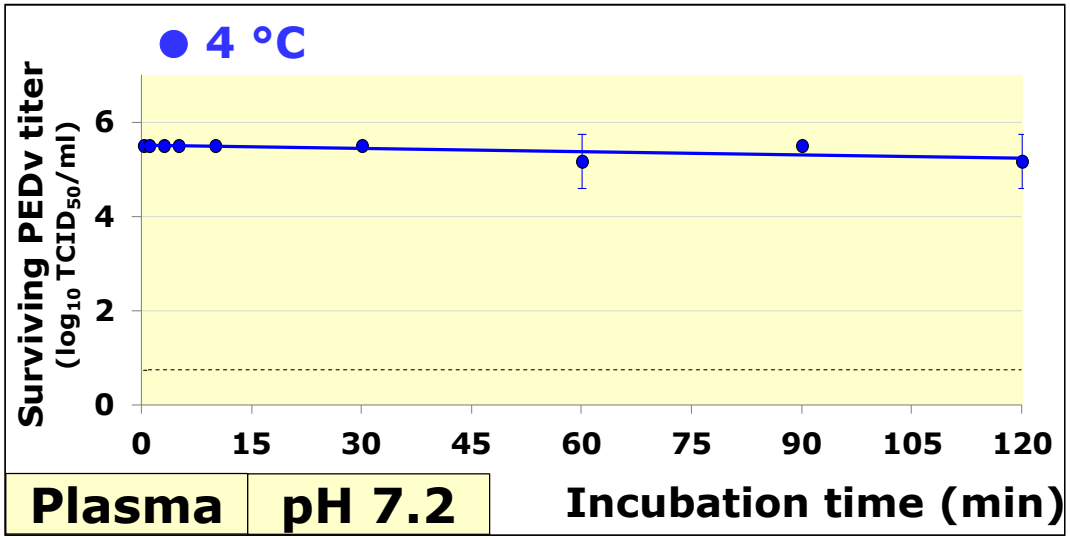
    1:10 in 175 cm² tissue culture flasks



Results & Discussion - Survival Curves

Spike-Inactivation Assay



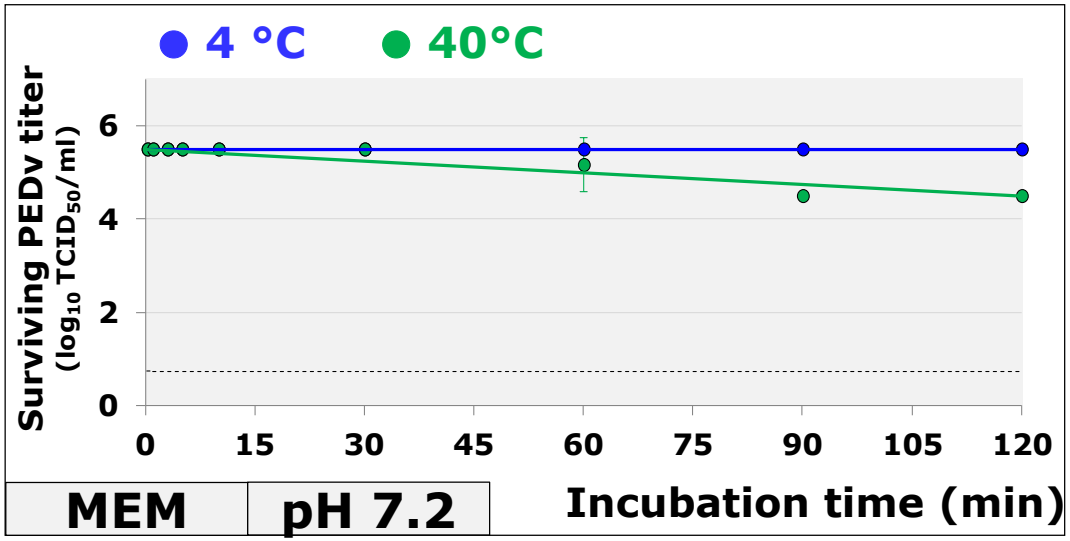


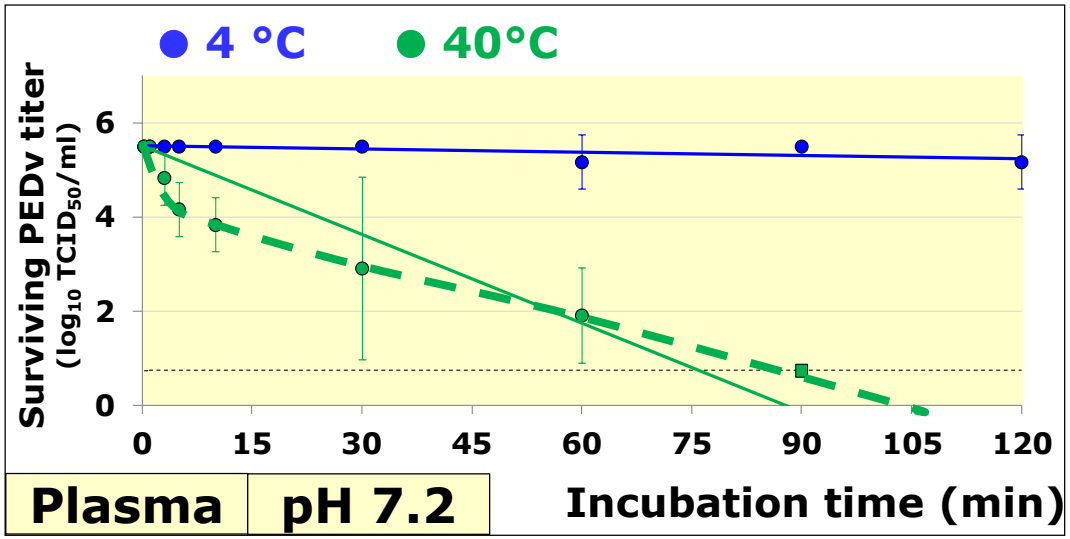
Sensitivity of PEDV

Stable in MEM at 4°C
Stable in plasma at 4°C

Results & Discussion - Survival Curves

Spike-Inactivation Assay





Sensitivity of PEDV

Stable in MEM at 4°C
Stable in plasma at 4°C

Stable in MEM at 40°C
Sensitive to 40°C in plasma (tailing effect in plasma)

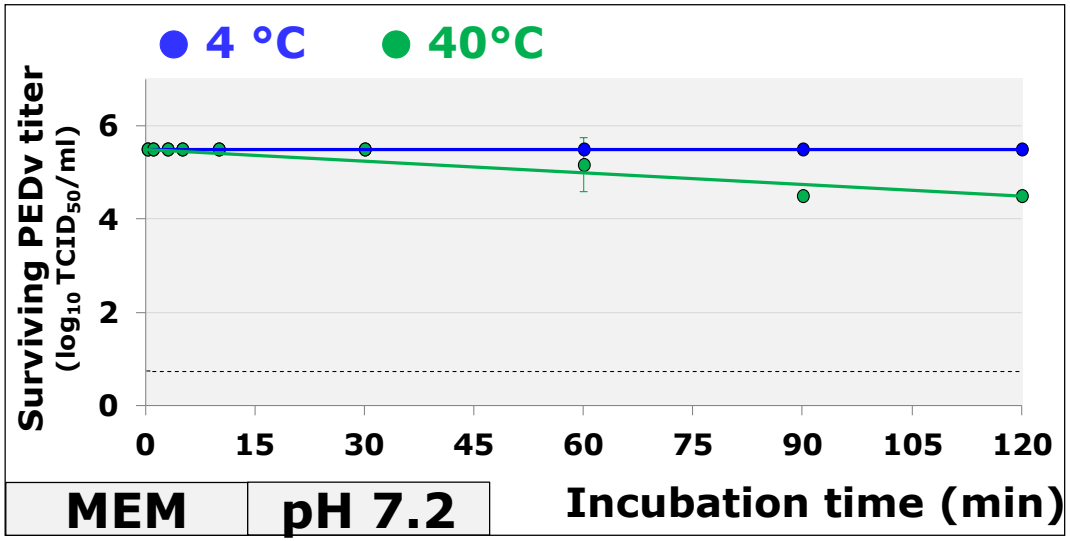
$D_{40°C,MEM}$ = 120 ± 17 min

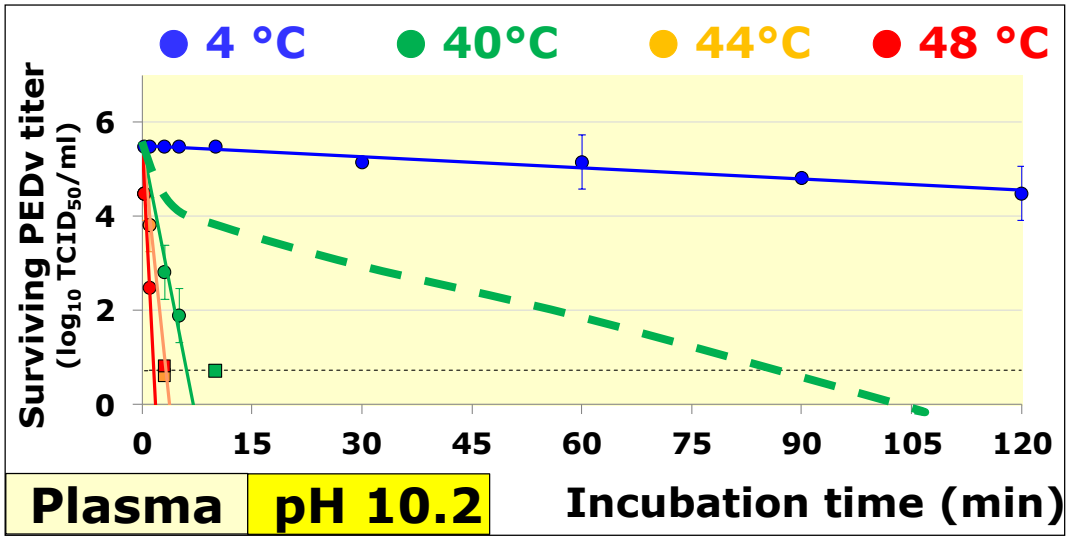
$D_{40°C,plasma}$ = 16 ± 5 min

7.5 x faster "in vivo" conditions

Results & Discussion - Survival Curves

Spike-Inactivation Assay





Sensitivity of PEDV

Stable in MEM at 4°C
Stable in plasma at 4°C

Stable in MEM at 40°C
Sensitive to 40°C in plasma (tailing effect in plasma)

$D_{40°C,MEM}$ = 120 ± 17 min

$D_{40°C,plasma}$ = 16 ± 5 min

7.5 x faster "in vivo" conditions

HAT-pasteurisation plasma

[48°C - pH 10.2 - 10 min]

D-plasma$_{UCL95}$: 35 sec

17.4 log PEDV TCID$_{50}$/ml

(in MEM: D_{UCL95}=114 sec → 5.3 log)

Quist-Rybachuck, Nauwynck, Kalmar, subm

Results & Discussion - Confirmation Assay

Acute phase serum has been reported to contain PEDV

Does PEDV remain infectious in plasma at *in vivo* conditions?

- ⇨ Spike-inactivation assay in tissue culture flasks
- ⇨ HAT determinants: H = 37°C; A = pH 7.2; **T = 120** or **T = 180 min**

D value at *in vivo* conditions

Matrix	Spike (log_{10} $TCID_{50}$)	Vol	pH	Temp (°C)	Time (min)	Surviving PEDV #	Measured D value (sec or min)	Expected D value mean	Expected D value [UCL95]
Plasma	5.80	1 ml	7.2	37	120	3	23.2 min	30.5	[55.8] min

Flask assay at *in vivo* conditions: $D_{37°C,\ pH\ 7.2}$ = 23.2 min

Even in absence of anti-PEDV IgG,
infective PEDV is inactivated in porcine plasma at *in vivo* conditions
(normal pig body temperature = 37.8-40°C)

Results & Discussion - Confirmation Assay

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obtainment of PEDV sterility at *in vivo* conditions

Matrix	Spike (log_{10} $TCID_{50}$)	Vol	pH	Temp (°C)	Time (min)	Surviving PEDV # PFU	Sterility obtained ? (yes/no)	Expected time to sterility mean	[UCL95]
Plasma	5.80	1 ml	7.2	37	180	0	YES	177	[324] min

Flask assay at *in vivo* conditions: $D_{37°C,\ pH\ 7.2}$ = 23.2 min

Even in absence of anti-PEDV IgG,
infective PEDV is inactivated in porcine plasma at *in vivo* conditions
(normal pig body temperature = 37.8-40°C)

***In vivo* conditions result in PEDV sterility**
of a 5.8 log_{10} $TCID_{50}$ per ml spike within 180 min

Conclusions

1. **Porcine plasma at body temperature shows anti-PEDV activity**
   - ❑ **but refrigerated plasma does not**
   - ❑ **tailing effect when inactivation at 37 - 40 °C**
   - ❑ **no tailing effect at 48 °C / important dilution effect (further trials)**

PEDV inactivation in seronegative porcine plasma at 37°C

- ➡ **± 0,44 million infectious particles /ml plasma (5.8 log $TCID_{50}$/ml)**
- ➡ **reduced to 3 (0) infectious particles in 2 h (3 h)**
- ➡ **viraemic PEDV would not remain infectious for prolonged times**

2. **Inactivation of PEDV is facilitated in plasma**

non-specific anti-viral acitivity of plasma increases safety of SDPP

3. **Viral inactivation assays should take matrix-effects into account**

4. **Biosafety measures: needed at all points of the distribution chain**

Acknowledgements - Questions

PEDV syncytium

